ON THE NON-IDENTITY OF *TRYPANOSOMA BRUCEI* (PLIMMER AND BRADFORD, 1899) WITH THE TRYPANOSOME OF THE SAME NAME FROM THE UGANDA OX*

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INTRODUCTION

Before considering our own observations it will be necessary to review briefly previous statements regarding the morphology of *Trypanosoma brucei*.

(1) Bruce[1] states that the haematozoa vary among themselves a good deal in shape and size and seem to take on slightly different forms in different species of animals. He publishes four figures depicting nine trypanosomes. Possibly one or two of the five figured from the dog might be considered to be 'stumpy' forms.

(2) Kanthack, Durham, and Blandford[2] state that the Nagana parasites vary considerably both in size and form. They may be long and pointed and sometimes stouter, some individuals are short and thick with a short flagellum, their protoplasm being crowded with granules. This description suggests dimorphism, but it should be noted that forms without a free flagellum are not mentioned. No slides were available belonging to these observers, but Dr. Durham kindly lent us a large series of photographs. On examining these, one or, perhaps, two show a 'stumpy' form, but it is difficult to be certain, and the uniformity of the remainder is striking. They state that 'the material for our observation was obtained in the first instance from the blood of a dog infected by

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the disease on the voyage from Africa, and brought to England in November, 1896, by Dr. Waghorn.'

This animal we believe to be the origin of the strain of *T. brucei*, Plimmer and Bradford, 1899, described by these authors, and at present maintained in England, so far as we can gather, solely at Liverpool.

It is not stated above from what animal the dog was infected on the voyage, nor is it stated what the exact original source of the strain derived from Zululand was.

(3) Plimmer and Bradford[3, 4] describe four forms in the blood, but neither their description nor figures suggest that they have seen stumpy forms. They describe 'a large hyaline form.' 'This organism is much larger than the ordinary adult form, and is much wider, often more than double the width, and is more irregular in shape. The protoplasm is quite homogeneous and much more delicate, and it stains very faintly with the methylene blue.' They are still to be found in films, and we easily found them in Dr. Plimmer's old films, but we found only extremely rarely forms that could be called 'stumpy.' We think that if they had been present these observers would hardly have failed to have noticed and drawn attention to them, as they have a striking appearance.

(4) Bruce and others[5] make a comparison between *T. brucei*, Uganda, 1909, and *T. brucei*, Zululand, 1894.

They state that many of the old Zululand preparations are still extant, so that it has been possible to do this. The preparations were, however, 15 years old, and had been stained with carbol fuchsin. The slides were got from horse, donkey, ox, monkey, dog; 200 trypanosomes were measured from the Zululand strain and 172 from the Uganda strain. The curves obtained in this way certainly resemble one another, though in one case the peak is at 18μ, in the other case at 20μ.

Also trypanosomes are figured from each strain, and there can be little doubt that there is a close resemblance, if not identity, viz., in the fact that both possess both long and stumpy forms. 'With the evidence available the Commission consider themselves justified in considering the trypanosome recovered from the Uganda ox to be identical with *T. brucei*, the cause of Nagana in Zululand and other parts of South Africa.'

Further, Bruce states in another paper[6] that *T. brucei* (Uganda strain) has actually 26 per cent. of non-flagellated forms.

(5) In 1911 Laveran[7] published an article, entitled 'Identification et essai de classification des trypanosomes des mammifères.'

In this article he places *T. brucei* in his Group I, 'Trypanosomes chez lesquels le flagelle présente toujours une partie libre,' whereas he places *T. gambiense* in his Group III, 'Trypanosomes ayant des formes à flagelle libre et des formes sans flagelle libre.'

Or, in other words, *T. brucei* is classed among the monomorphic trypanosomes while *T. gambiense* is among the dimorphic. We have then two opposite statements as to the morphology of *T. brucei*, (1) that it is monomorphic and (2) that it is dimorphic.

We possess two strains of (so-called) *T. brucei* in the laboratory, viz., the Zululand strain, of which we have given the origin above, and the Uganda strain from Surgeon-General Bruce, obtained originally from the ox in Uganda in 1909. These strains have been maintained continuously at Runcorn in a variety of animals, the Zululand strain for $4\frac{1}{2}$ years, and the Uganda strain for $2\frac{1}{4}$ years. In the case of the Uganda strain it was lost in 1912 for a short period but was returned to us again by Prof. Mesnil, who had previously received it from us.

We made then a preliminary examination of these two strains, and found to our surprise that they could easily be distinguished morphologically.

We next proceeded to make a detailed examination of the two strains in a series of slides throughout the entire period of infection in various animals, viz., rats, guinea-pigs, and rabbits. As the result, we believe we have established the following facts:—

(*a*) The Zululand strain is *typically monomorphic*. The trypanosomes are long, with a long free flagellum. We must admit, however, that it is possible (as we believe is the case also in another typically monomorphic trypanosome, viz., *T. evansi*), to find by long search short forms which somewhat resemble true stumpy forms, but we must emphasise the fact that in all the slides we have examined, prolonged search is necessary to find them.

(*b*) We have also verified the fact that Laveran's *T. brucei* strain also is, as he says, monomorphic. The origin of this strain seems uncertain. Laveran probably received it from Ehrlich, but

where the latter got it from cannot now be ascertained. Unless it came from England, there must be two monomorphic *T. brucei* strains in existence, not to mention the possibility of other *T. brucei* strains of uncertain parentage in various laboratories.

We have examined also old slides from the Zululand strain lent us by Prof. Nuttall, Colonel Skinner, R.A.M.C., and Dr. Plimmer.* All these were monomorphic. We repeat here that in these films, or at least some of them, it was possible by long search to find a short form somewhat resembling a stumpy form, but not having the somewhat indefinable characteristic appearance of the latter.

(*c*) The Uganda strain, on the contrary, is *typically dimorphic*, i.e., besides the usual long forms of trypanosomes, stumpy forms are readily found, even in abundance occasionally, when the infection is well marked. Bruce,[6] as we have noted above, states that this trypanosome has 26 per cent. of non-flagellated forms.

The typical stumpy form we may define as a short, thick trypanosome, 12-14μ, almost straight or slightly curved along one edge, while along the other the membrane is thrown into bold folds, there being no free flagellum, or at times a very short or doubtfully free one.

It is thus easy to distinguish a typical Uganda specimen from a Zululand specimen, and, in fact, we may express the difference in this way, that it is impossible to match a typical Uganda slide by any slide from the Zululand strain.

We have stated in the above history of the Uganda strain that it was recently returned to us by Prof. Mesnil, who remarked in his letter that he had maintained it in mice (for nearly a year), and that it showed now very few 'trapues' forms. This we have been able to verify in the films made from the infected mouse sent to us. But, as soon as we had re-inoculated it into guinea-pigs, it again showed numerous stumpy forms. But the same does not hold good for the Zululand strain; in guinea-pigs, as in rats and rabbits, the strain is *typically monomorphic*, i.e., it does not show stumpy forms. We therefore conclude that the two strains, as we now possess them in the laboratory, are different.

* We desire here to express our thanks to these gentlemen for their kindness in sending us slides.

How, then, are we to explain these facts? There seem to us three possibilities:—

1. That the strain we now possess, which we have been designating *T. brucei*, Zululand, is not this strain at all, but some other trypanosome inoculated erroneously during the course of inoculations extending over years. We think this view is untenable, for it would not explain the monomorphic character of the old slides we have examined, nor would it explain Laveran's monomorphic trypanosome.

2. While Bruce may have been working with a dimorphic trypanosome in Zululand, and still has slides showing these characters, it is quite possible that the strain sent by him to England was something quite different. This is all the more likely, as Bruce successfully infected dogs from a variety of wild game, viz., wildebeeste, kudu, bush buck and buffalo, and, as Bruce himself states, 'when *T. brucei* was discovered in Zululand in 1894, it was naturally thought to be the one and only trypanosome in Africa,' and no suspicion arose at that time of a multiplicity of trypanosomes in native game.

This is the simplest explanation, and the fact that Plimmer and Bradford do not describe or figure stumpy forms, and our examination of Dr. Plimmer's slides had the same result, makes it probable that this is the true one.

3. That the strain originally sent to England was dimorphic, but that it has now become monomorphic. This may have come about in two ways:—

(*a*) The strain originally was a *mixture* of a long trypanosome and a stumpy trypanosome, and the stumpy has now died out. If this explanation were valid, it would probably imply that *T. gambiense* and other dimorphic trypanosomes were also mixtures. This we regard as a not impossible view, but one we cannot at present prove or disprove.

(*b*) The strain was originally *dimorphic* (but not a mixture), and that it has now become *monomorphic*. If this were so, it would modify materially our notions of specificity of trypanosomes, at least in laboratories. Of such a change we have at present not much evidence. We have noted, however, above that the Uganda strain

kept in mice for a year was almost (but not entirely) monomorphic, but that in guinea-pigs it at once showed its normal characters.

It is impossible at present to decide between these explanations.

We come back, therefore, to the fact of which we ourselves have no doubt, viz., that the trypanosome that Plimmer and Bradford worked with, and which they named *T. brucei* in 1899, is certainly now a monomorphic trypanosome, and is not the same as the trypanosome from the ox described under the same name by Bruce and others in Uganda.

We believe, then, that the facts we have brought forward prove the non-identity of the Zululand and Uganda strains.

In order to avoid confusion, we think it advisable that this Uganda trypanosome should be re-named. We therefore propose for it the name *T. ugandae*.

REFERENCES

1. Bruce. 'Further Report on the Tsetse-fly Disease or Nagana in Zululand,' by Surgeon-Major David Bruce, A.M.S. (1896 ?). Harrison & Sons, London.

2. Kanthack, Durham & Blandford. Roy. Soc. Proc., 1898, Vol. LXIV, p. 100.

3. Plimmer & Bradford. 'A Preliminary Note on the Morphology and Distribution of the Organism Found in the Tsetse-fly Disease,' Roy. Soc. Proc., 1899-1900, Vol. LXV, p. 274.

4. Bradford, J. R., & Plimmer, H. G. 'The *Trypanosoma brucei*, the Organism Found in Nagana or Tsetse-fly Disease,' Quart. Journ. Micro. Sci., 1902, Vol. XLV, p. 449.

5. Bruce & Others. Reports of the Sleeping Sickness Commission of the Royal Society, 1911, No. XI, p. 147.

6. Bruce. *Ibid.*, 1912, No. XII, p. 24.

7. Laveran. 'Identification et essai de classification des trypanosomes des mammifères,' Ann. de l'Institut Pasteur, July, 1911, No. 7, p. 497.